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Characterization of a Bacteriophage That Carries the Genes for Production of Shiga-Like Toxin 1 in *Escherichia coli*

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The Shiga-like toxin 1-converting bacteriophage H-19B was recently shown to carry the structural genes for the toxin and was shown to have DNA sequence homology with phage lambda. We present evidence that the linear genome of bacteriophage H-19B has cohesive termini which become covalently associated during prophage integration. Integration occurs through a site on a 4-kilobase-pair *Eco*RI fragment located near the center of the bacteriophage chromosome. The relationship between bacteriophages H-19B and lambda was examined by Southern hybridization. Homologous regions were mapped on the respective chromosomes which corresponded to the regions of the *J* gene, the *int-xis* area, and the *O* and *P* genes of phage lambda. The H-19B *tox* genes were mapped to the right of the *O* and *P* gene homology, which was far away from the phage attachment site. We concluded that H-19B is a lambdoid bacteriophage. Unlike other toxin-converting bacteriophages, the toxin genes were not located adjacent to the phage attachment site. It appeared that the Shiga-like toxin 1 genes were not picked up by a simple imprecise prophage excision. H-19B could, however, have acquired chromosomally located toxin genes by a series of events involving deletion and duplication followed by aberrant excision.

Although toxigenic conversion of gram-positive bacteria such as *Corynebacterium diphtheriae* and *Staphylococcus aureus* has been well documented (1, 11, 14), such a phenomenon has only recently been demonstrated in gram negative organisms. Smith et al. (23) have reported that several strains of Shiga-like toxin 1 (SLT-1) producing *Escherichia coli* harbored a bacteriophage which could mediate the transfer of the toxinogenic property to *E. coli* C600. O'Brien et al. (20) have confirmed this finding and have shown that the phage mediates high-level SLT-1 production. Furthermore, they isolated from an *E. coli* O157:H7 strain another highly related bacteriophage designated 933J, which mediated toxin conversion. We have shown recently (12) that one of the converting phages designated H-19B, which was isolated by H. Williams Smith, carries the structural genes for the two *E. coli* SLT-1 subunits. Newland et al. (19) have reported almost identical results for the SLT-1 genes of bacteriophage 933J. In this report we present data which show that phage H-19B has cohesive termini and integrates into the *E. coli* chromosome via a site on a centrally located 4-kilobase-pair (kbp) *Eco*RI fragment. There was significant homology between H-19B DNA and the restriction fragments of lambda that carry the *J* gene, the integration functions, and the genes for DNA replication. The SLT-1 genes did not appear to be located close to the attachment site on the phage chromosome.

MATERIALS AND METHODS

Strains and media. *E. coli* C600, which carries the toxin-converting phage H-19B, was received from H. Williams Smith. *E. coli* TB1 [*lac pro rpsL ara thi* φ80d(*lacZ*) M15 *hsdR*] was obtained from Besthesda Research Laboratories (Gaithersburg, Md.) and was used as the host for the pUC vectors and recombinant plasmids. Plasmids pUC9, pUC18, and pUC19 were obtained from J. Messing and J. Vieira (16). Phage lambda DNA was obtained from Boehringer Mannheim Biochemicals (Canada). This DNA was prepared from the inducible mutant *c*I ts857 *S*am7. Strains were grown in L medium (17) or brain heart infusion supplemented as necessary with carbenicillin (50 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (50 μg/ml; Boehringer Mannheim).

DNA preparation. Bacteriophage H-19B DNA was prepared from purified phage stocks by a method used for the purification of phage lambda DNA (15). Low-titer phage stocks were prepared from overnight cultures of the lysogen *E. coli* C600 (H-19B) in brain heart infusion supplemented with 3 mM calcium chloride and 3 mM magnesium chloride. High-titer phage stocks were prepared and purified as described previously (12). Plasmid DNA ws prepared by the method described by Birnboim and Doly (2). Further purification was achieved by ultracentrifugation in cesium chloride-ethidium bromide gradients (15). Plasmid DNA was electrophoresed in 0.7 to 1.5% agarose gels with Tris borate-EDTA buffer (15).

Restriction mapping and cloning. Restriction endonucleases were purchased from Boehringer Mannheim, and digestions were performed by following the instructions of the manufacturer. Phage lambda DNA digested with *Hin*dIII or *Eco*RI-*Hin*dIII was used as a molecular weight standard (13, 21). Restriction mapping was performed by a variety of methods, including double digestion, partial digestion, and digestion of isolated fragments. Conclusions sometimes were confirmed by hybridization. In most cases restriction fragments were extracted from low-melting-temperature agarose gels (Bio-Rad Laboratories, Richmond, Calif.), as described by Maniatis et al. (15). Ligations were carried out at 14°C with 1 U of T4 ligase per 20-μl reaction.

Southern blotting and hybridization. Southern blotting and hybridizations were performed in the standard manner (24). Hybridizations were performed at 37°C in 50% formamide and 1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium

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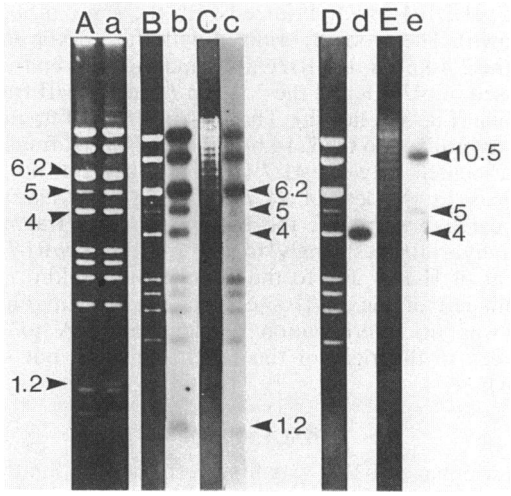

FIG. 1. Cohesive termini and localization of the phage *att* site of H-19B. Lane A, *Eco*RI digest of H-19B; lane a, *Eco*RI digest of H-19B that was heated and cooled before electrophoresis; lane B, *Eco*RI digest of H19B; lane C, *Eco*RI digest of strain C600 (H-19B) lysogen; lanes b and c, autoradiograms of hybridization with labeled H-19B DNA; lane D, H-19B *Eco*RI; lane E, C600 (H-19B) *Eco*RI; lanes d and e, autoradiograms of hybridization with labeled pJLB101. Fragment sizes for each experiment are indicated (in kilobase pairs) by arrows.

citrate) with 10× Denhardt solution (7). After hybridization, filters were washed with 1× SSC at 68°C for 1 h. These conditions represent T_m minus 17°C for phage lambda DNA (5). Information regarding restriction sites and coding regions for phage lambda was taken from previously published data (13, 21).

RESULTS

The results of gel electrophoresis patterns of H-19B DNA digested with *Eco*RI without heating and cooling are shown in Fig. 1, lane A. DNA was heated to 70°C and cooled rapidly on ice before electrophoresis to dissociate fragments associated by hydrogen bonding (Fig. 1, lane a). In this case bands with lengths of 1.2 and 5.0 kbp in lane a were more intense than those in lane A. A fragment resulting from the noncovalent association of these fragments and with a length of 6.2 kbp was more intense in lane A than in lane a. *Hin*dIII fragments of 7 and 22 kbp and *Sal*I fragments of 2.3 and 7.5 kbp were also found to reversibly associate (data not shown).

To determine whether phage H-19B integrates into the *E. coli* chromosome, we hybridized ^{32}P-labeled total phage DNA with filter-bound *Eco*RI-digested DNA of the purified phage, total chromosomal DNA isolated from the strain C600 (H-19B) lysogen, or C600 chromosomal DNA. Before the gel was loaded, all samples were heated to 70°C and cooled on ice to disrupt noncovalently associated cohesive termini. Fragments of 1.2, 5.0, and 4.0 kbp, which can be seen in the phage digest (Fig. 1, lanes B and b), were not visualized by hybridization in the lysogen (Fig. 1, lane c), although all other phage bands were. Phage DNA did not hybridize to strain C600 chromosomal digests (data not shown). To visualize the new fragments that would be expected to be generated at the prophage termini, we used a more specific probe, pJLB101, which carried the 4-kbp

TABLE 1. Plasmid constructions used for hybridizations

Designation	Insert	Vector
pJLB101	H-19B, 4-kb *Eco*RI (fragment E)	pUC18
pJLB102	H-19B, 2.7- and 0.9-kb *Eco*RI (fragments L and G)	pUC9
pJLB100	H-19B, 3.0-kb *Hin*dIII (fragment E)	pUC18
pJLB4	H-19B, 2.7-kb *Hin*dIII *Eco*RI (from *Eco*RI fragment B)	pUC9
pJLB104	Lambda, 6.6-kb *Hin*dIII (fragment C)	pUC18

H-19B *Eco*RI fragment cloned in pUC18 (Table 1). In a previous study (12) it has been shown that there is no homology between H-19B and pUC18. The probe hybridized to a 4-kbp fragment in the phage digest and to 5.5- and 10.5-kbp fragments in the lysogen is shown in Fig. 1 (lanes d and e, respectively. The location of the 4-kb *Eco*RI fragment of H-19B which carries the phage attachment site is shown on the map in Fig. 2.

The sizes of fragments cleaved from H-19B by restriction enzymes are presented in Table 2. The ordering of the *Eco*RI fragments in the 11-kbp *Hin*dIII B fragment of phage H-19B differed from that presented previously (12). The mapping was originally done by examining *Eco*RI partial digests of the isolated *Hin*dIII fragment. In this study the recombinant plasmids pJLB101 and pJLB102, which carry the *Eco*RI E fragment and both the *Eco*RI L and G fragments, respectively (Table 1), were used as probes in hybridizations to Southern blots of H-19B DNA that had been partially digested with *Eco*RI. The results were compatible with the order E, H, I, L, G, (Fig. 2; data not shown). The two *Sal*I sites and the positions of *Hin*dIII fragments F and G are also shown in Fig. 2. Further details of the construction of the map have been presented elsewhere (A. Huang, M.S. thesis, University of Toronto, Toronto, Ontario, Canada, 1986).

^{32}P-labeled H19-B chromosomal DNA was hybridized to Southern blots of lambda DNA that was cleaved with several restriction enzymes. Representative hybridizations are shown in Fig. 3A, and the results are summarized (Fig. 3B). H-19B hybridized to the 23-kbp *Hin*dIII fragment on the left arm of lambda. The area of homology was localized to the region between the *Hpa*I site at 14.99 kbp and the *Ava*I site at 19.39 kbp since there was no hybridization to the *Ava*I D fragment or *Hpa*I fragments J, H, and G, which lie between

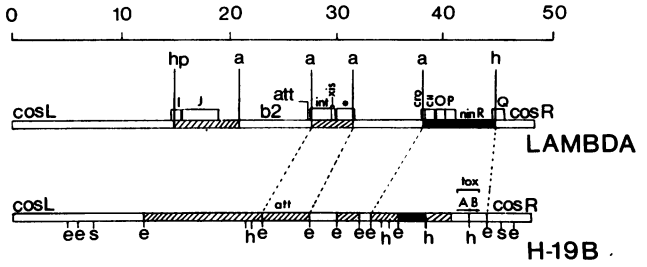

FIG. 2. Restriction endonuclease maps of H-19B and lambda (showing only relevant restriction sites). Areas of homology that were shown to be specific are joined by broken lines. Abbreviations: hp, *Hpa*I; a, *Ava*I, e, *Eco*RI; h, *Hin*dIII; S, *Sal*I; asterisk indicates coding region for the open reading frames Ea 8.5 and Ea 22 and 265 bp of the *exo* gene. The order of H-19B fragments for *cosL* is as follows: *Eco*RI fragments D, K, C, A, E, H, I, L, G, B, F, and J; *Hin*dIII fragments A, G, B, F, D, E, and C; and *Sal*I fragment B, A, and C.

TABLE 2. Restriction fragment sizes of H-19B

Fragments (size [kbp]) of the following restriction enzymes:		
*Sal*I	*Hin*dIII	*Eco*RI
A (37)[a]	A (22)[a,b]	A (11.3)
B (7.5)[b]	B (11.5)	B (8.1)
C (2.3)[b]	C (7.0)[b]	C (6.2)
	D (4.0)	D (5.0)[b]
	E (3.0)	E (4.0)
	F (0.7)	F (3.0)
	G (0.4)	G (2.7)
		H (2.5)
		I (2.0)
		J (1.2)[b]
		K (1.0)
		L (0.9)

[a] Size estimated as sum of *Eco*RI fragments.
[b] Fragments carried cohesive termini.

cosL and 14.99 kbp (Fig. 3A, lanes b and c). *Hpa*I fragments K, L, and N were all less than 700 bp in length and were not visualized adequately to rule out hybridization. H-19B DNA hybridized to the 14.6-kbp *Ava*I A fragment that lies to the left of 19.39 kbp but not to the 1.6- and 6.9-kbp I and C fragments that lie to the right (Fig. 3A, lane c).

There was no hybridization to fragments in the b2 region (*Hin*dIII-E and -F, *Ava*II-C, and *Hpa*I-C). In the central region, H-19B DNA hybridized to the 9-kbp *Hin*dIII B fragment. The homology was confined to the 3.7-kbp *Ava*I fragment lying between 27.8 and 31.6 kbp since there was no hybridization to the two *Ava*I fragments (G and E) lying between 31.6 and 39.9 kbp. The cloned 4.0-kbp *Eco*RI fragment of H-19B DNA was homologous only to the 3.7-kbp *Ava*I fragment of lambda DNA (Fig. 3A, lane e).

There was strong hybridization to the 6.6-kbp *Hin*dIII fragment on the right arm of the lambda genome but none to the 4.0-kbp *Hin*dIII fragment which carries *cosR* (Fig. 3A, lane a). The cloned 8.1-kbp *Eco*RI fragment of H-19B, which bears the SLT-1 structural genes (12), hybridized only to the 6.6-kbp *Hin*dIII fragment of lambda (data not shown). In reciprocal hybridizations, labeled lambda DNA hybridized to *Eco*RI fragments of H-19B DNA with lengths of 11, 8.1, 4.0, and 2.7 kbp. There was also minimal hybridization to the 2.0-kbp fragment (Fig. 3A, lane d). The results are summarized in Fig. 2. The homologous regions are located in relation to the phage genetic map drawn from the nucleotide and deduced amino acid sequences presented by Kessler et al. (13) and Sanger et al. (21).

The homologous regions on the right arms of lambda and H-19B were examined in more detail. The recombinant plasmid pJLB102, which contains the 0.9- and 2.7-kbp *Eco*RI fragments of H-19B, hybridized only to the 1.7-kbp *Hin*dIII-*Eco*RI fragment of lambda DNA located between 37.45 and 39.17 kbp (Fig. 3A, lane f). In reciprocal hybridization, there was no homology between lambda and the 0.9-kbp H-19B *Eco*RI fragment (data not shown). The 2.7-kbp *Eco*RI-*Hin*dIII fragment of H-19B was isolated from pJLB4; nick translated; and hybridized to *Eco*RI, *Sst*II, and *Bam*HI-*Sst*II double digests of pJLB104, which carries the *Hin*dIII C fragment of lambda (Fig. 4A, lanes A, a and B, b, respectively). This probe hybridized to the 1.22-kbp *Eco*RI-*Sst*II fragment of lambda and to the *Sst*II-*Hin*dIII fragment (3.7 kbp), but not to the 4.2-kb fragment composed of the 1.7-kbp *Hin*dIII-*Eco*RI fragment of lambda and the pUC18 vector (Fig. 4A, lane a). There was no hybridization to the 1.7-kbp *Hin*dIII-*Eco*RI fragment when a *Hin*dIII-*Eco*RI digest of pJLB104 was hybridized with the same probe (data not shown). The probe hybridized with the 1.3-kbp *Bam*HI-*Sst*II, the 2.4-kbp *Bam*HI-*Hin*dIII, and the 5.4-kbp fragment composed of pUC18 and the 2.9-kbp *Hin*dIII-*Sst*II fragment of lambda (Fig. 4A, lane b). The 3.0-kbp *Hin*dIII fragment of H-19B hybridized to the 2.4-kbp *Bam*HI-*Hin*dIII fragment of lambda located between 41.7 and 44.14 kbp but not to the sequences to the left of 41.7 kbp (Fig. 4A, lane c). In reciprocal hybridization, the 6.6-kbp *Hin*dIII fragment of lambda hybridized strongly to the 2.7-kbp *Eco*RI-*Hin*dIII fragment of H-19B and to the area of the 3.0-kbp *Hin*dIII fragment left of the *Bgl*II site (data not shown; Fig. 4B). There was no hybridization of lambda DNA to H-19B sequences to the right of the *Bgl*II site (data not shown; Fig. 4A).

DISCUSSION

Bacteriophage H-19B was first described by Smith et al. (23) as a SLT-1-converting bacteriophage isolated from the SLT-1-producing *E. coli* H-19 serogroup O26. In this report we have described a more detailed study of phage H-19B. The results of hybridization of labeled phage to digests of DNA extracted from the uninduced lysogen are consistent

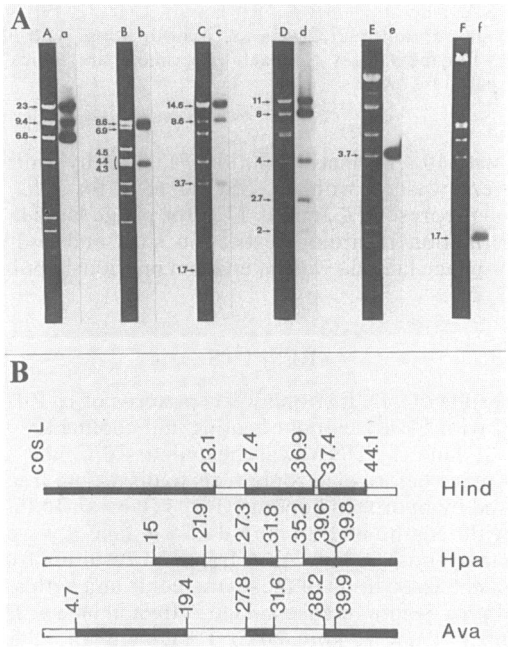

FIG. 3. (A) Hybridization of H-19B and lambda DNA. Lambda DNA was digested with *Hin*dIII (lane A), *Hpa*I (lane B), and *Ava*I (lane C). Lanes a, b, and c, autoradiograms after hybridization with labeled H-19B DNA; lane D, H-19B *Eco*RI; lane d, autoradiogram of hybridization with labeled lambda DNA; lane E, lambda *Ava*I; lane e, hybridization with pJLB101; lane F, lambda *Eco*RI and *Hin*dIII; lane f, hybridization with pJLB102. (B) Homologous areas on lambda chromosome. Open bars indicate no homology, and solid bars indicate homology demonstrated on Southern hybridizations. Restriction sites bounding homologous fragments are given (in kilobase pairs) from *cosL*. Sites in nonhomologous areas are not shown. The order and size of lambda fragments (in kilobase pairs) from *cosL* are as follows. For *Hin*dIII fragments: A, 23.1; F, 2.04; E, 2.3; B, 9.4; G, 0.56; C, 6.68; D, 4.36. For *Hpa*I fragments: K, 0.7; D, 4.5; L, 0.4; J, 2.2; N, 0.25; H, 3.38; G, 3.40; B, 6.9; C, 5.4; E, 4.49; M, 0.4; I, 3.0; F, 4.34; O, 0.22; A, 8.66. For *Ava*I fragments: D, 4.7; A, 14.6; I, 1.60; C, 6.88; F, 3.73; G, 1.88; E, 4.71; H, 1.67; B, 8.61 (12).

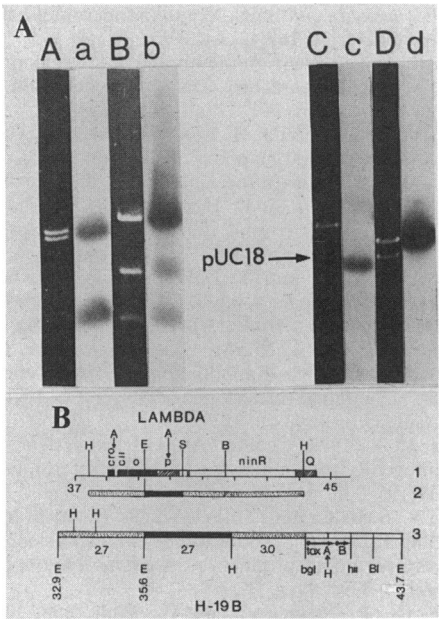

FIG. 4. (A) Hybridization between the 6.6-kbp *Hin*dIII fragment of lambda (carried in recombinant plasmid pJLB104) and regions of the 8.1-kbp *Eco*RI fragment of H-19B. Lane A, pJLB104 *Eco*RI-*Sst*II; lane B, pJLB104 *Bam*HI-*Sst*II; lanes a and b, hybridization with labeled 2.7-kbp *Eco*RI-*Hin*dIII fragment from H-19B (isolated from pJLB4); lane C, pJLB104 *Hin*dIII-*Bam*HI; lane D, pJLB100 *Hin*dIII; lanes c and d, hybridization to labeled 3.0-kbp *Hin*dIII fragment of H-19B (isolated from pJLB100). In pJLB104 the *Hin*dIII site at 44.14 kbp in lambda is adjacent to the *Bam*HI and *Eco*RI sites of the multiple cloning site of the pUC18 vector. (B) Map of lambda and H-19B showing homologous areas in right arms. Line 1, Lambda map showing coding regions (solid, hatched and closed bars; line 2, representation of homology of H-19B to lambda of *Hin*dIII-*Eco*RI, *Sst*II-*Bam*HI, and *Bam*HI-*Hin*dIII fragments (speckled boxes) and *Eco*RI-*Sst*II (solid boxes); line 3, map of H-19B with location of *tox* genes and homology to lambda of 2.7-kbp *Eco*RI, 2.7-kbp *Eco*RI-*Hin*dIII, and 2.3-kbp *Hin*dIII-*Bgl*II fragments. Abbreviations: H, *Hin*dIII; A, *Ava*I; E, *Eco*RI; S, *Sst*II, B, *Bam*HI, bgl, *Bgl*II; hll, *Hin*cII; Bl, *Bal*I. Map positions are in kilobase pairs from the *cosL* gene of lambda and H-19B.

with the Campbell model of phage integration in which the cohesive termini of the linear genome are covalently associated and the circle is integrated into the chromosome by a site-specific reciprocal crossover. The H-19B bacteriophage attachment site was found to be located on the central 4-kbp *Eco*RI fragment. The difference in the intensity of the hybridization signals to the 10.5- and 5-kbp bands shown in Fig. 1 (lane e) suggests that the attachment site lies close to one end of the 4-kbp fragment.

The areas of homology between the genomes of H-19B and lambda are summarized in Fig. 2. The homologous sequences are located in the same general areas of the respective maps, suggesting that the organization of the genomes is similar. Although we did not show that the homology in the left arm of lambda was specific for the left arm of phage H-19B, we presume that it was because the central and right arm areas were specifically homologous when smaller probes such as the 4- and 8.1-kbp *Eco*RI fragments of H-19B were used. By using the DNA sequence of phage lambda published by Kessler et al. (13) and Sanger et al. (21), it was possible to map the location of lambda coding sequences in relation to restriction sites with complete certainty (Fig. 2 and 4). The region of homology between the *Hpa*I and *Ava*I sites in the lambda left arm carries 400 bp of the *I* gene, the complete *J* gene (3.7 kbp), and 400 bp of the b2 region. Although the homology was not defined more precisely, it seems likely that it is confined to the *J* and possibly the *I* coding sequences, because results of studies of lambdoid phages have shown that at least part of the *J* gene, but not the b2 region, is conserved (8, 22, 25).

In the center of the genomes, the 4-kbp H-19B *Eco*RI fragment that carries the phage attachment site was found to be homologous to an *Ava*I fragment, about 50% of which encodes the lambda integrase and excisionase. The remainder of this lambda fragment carries two open reading frames of uncertain significance designated Ea 8.5 and Ea 22, as well as 265 bp of the *exo* gene (21). The data do not permit us to determine with certainty which of these areas is homologous.

The 2.7-kbp *Eco*RI fragment of H-19B was homologous to a fragment of lambda carrying the *c*I, *cro*, *c*II, and 60% of the 5′ end of the *O* gene (Fig. 4B). The 2.7-kbp *Eco*RI-*Hin*dIII fragment of H-19B was homologous to the *Eco*RI-*Sst*II fragment of lambda that carried 40% of the 3′ end of the *O* gene, the entire *P* gene, and a few hundred base pairs of the *ren* gene. We suspect that the *Eco*RI site at 36 kbp on the H-19B map is located in the *O* gene homolog, as is characteristic of lambdoid phages (9, 10, 18). This must, however, be confirmed by DNA sequencing. There is some homology between the 2.7-kbp *Eco*RI-*Hin*dIII fragment, as well as the 3.0-kbp *Hin*dIII fragment of H-19B, and the nonessential *ninR* region of lambda. Phage H-19B appears to differ from lambda with respect to assembly and structural genes. This is not surprising in view of the different morphology of the head noted by O'Brien et al. (20) and in our unpublished observations (Huang, M.S. thesis). The late genes and terminase are also unrelated.

Lambdoid phages have been defined on the basis of their ability to grow in *E. coli* and undergo homologous recombination with phage lambda, or on the basis of the cohesiveness of their termini with those of lambda (4). A central feature of lambdoid phages is that their genomes are organized in a functionally homologous manner (4). At the DNA sequence level, heteroduplexes of lambdoid phages have shown that the majority of sequences in the b2 region of lambda are not conserved (8, 22). There is extensive conservation of structural and assembly genes in lambda, 434, φ82, and φ80, while only about 50% of these sequences are related in φ21. There appears to be conservation of the 5′ end of the *J* gene in lambda, 434, φ82, and φ21 but not in φ80 (8, 22). Significant homology in the *O* and *P* genes of lambda, 434, φ21, and, to a lesser degree, φ80 has been demonstrated (8, 10, 18, 22). It has been shown that mutants in the *P* genes of lambdoid phages can be complemented but that *O* gene function appears to be phage specific (10, 18). Thus, functional specificity occurs despite sequence homology detected by electron microscopic heteroduplex methods. We have not attempted to demonstrate homologous recombination between H-19B and lambda. The relatively large fragments used in our hybridization experiments made it impossible to assign homology to single cistron sequences. Nor was it possible with our results to predict the capacity of H-19B and lambda to undergo homologous recombination. Nevertheless, the distribution of the homologous regions strongly suggests that the H-19B genome is organized in a fashion characteristic of lambdoid phages and that H-19B should be classified as such.

The toxin-converting corynephages of *Corynebacterium*

diphtheriae (11, 14) and *Staphylococcus aureus* enterotoxin A-converting phages (1) have been shown to carry the toxin structural genes in close association with the phage attachment site. This arrangement is commonly found in lambda *bio* and *gal* specialized transducing phages and suggests that a primordial chromosomal toxin gene became part of the phage genome by a simple imprecise prophage excision event (25). While one could hypothesize that the SLT-1 genes were inserted into H-19B by transposition, specialized transducing lambda phages which carry chromosomal genes between the *P* and *Q* genes have been reported (3). λ *gal* M3 and λ *aroG gal* are two examples that were produced in a three-step process involving deletion of prophage and adjacent chromosomal material to approximate the *gal* and *Q* genes. Insertion of a λ prophage adjacent to the deleted prophage followed by an aberrant excision in the first instance produced a phage carrying *gal* in place of *Q* (λ *gal* M3), and in the second instance produced *aroG gal* located between the *P* and *Q* genes (λ *aroG gal*) (3). The arrangement of the SLT-1 genes in H-19B is analogous to the latter example and suggests that the SLT-1 genes could originally have been picked up from the *E. coli* chromosome. It will be interesting to determine whether the toxin cistrons are localized in the same region in all SLT-1-converting phages or whether they are adjacent to the phage attachment site in some cases.

It is apparent from the results shown in Fig. 2 that by comparison with lambda, the *tox* genes probably lie in the putative rightward operon of H-19B. Results of transcriptional studies reported in the accompanying paper (6) indicate that the direction of transcription of the SLT-1 operon is the same as that for the putative rightward operon of phage H-19B. It is unclear whether such concordance has significance in the regulation of toxin biosynthesis or in the biology of the phage.

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